COLONY GROWTH AND PATTERN IN THE TWO-TENTACLED HYDROID, PROBOSCIDACTYLA FLAVICIRRATA

RICHARD D. CAMPBELL [1]

Friday Harbor Laboratories, University of Washington, and Department of Organismic Biology, University of California, Irvine

Proboscidactyla flavicirrata is a species of hydroid whose colony is intimately patterned around its commensal host, a sabellid worm. The hydroid colony covers the distal end of the leathery worm tube, and the polyps interact with the worm during feeding (Uchida and Okuda, 1941; Hand and Hendrickson, 1950). The colony pattern has been described by several workers (Hand, 1954; Brinckmann and Vannucci, 1965) but there is virtually nothing known about the colony development.

The observations presented in this paper indicate that the colony growth of *Proboscidactyla flavicirrata* is dynamic and approaches a steady condition. The colony expands as the worm elongates its tube distally. The colony degenerates in regions distant from the tip. The various axial regions of the stolon mat therefore represent successive ages of tissue. This growth pattern results largely from the unusual ability of the feeding polyps to move along the tube, keeping pace with tube elongation and generating a stolon system behind them as they advance.

Methods and Materials

Colonies of *Proboscidactyla flavicirrata* (Brandt) were collected during the summer months from large sabellid tubes (over 5 mm. in diameter) growing on the town floats at Friday Harbor, Washington. During the winter months colonies were kindly collected by Dr. Robert Fernald. Observations on colony and polyp progressions were made on material kept in a running sea water table, with the polyps fed every two days on sabellid eggs.

Polyps were explanted on microscope slides by placing them, with a small portion of attached stolon, upright on the surface. In grafting experiments, polyp segments were isolated with a scalpel and strung together on glass fibers to heal for three hours.

The terms *distal* and *proximal* refer to portions of the worm tube corresponding to the worm's anterior and posterior directions, respectively; the open end of the worm tube is distal. The term forward is used in reference to gastrozooid orientation, forward being that direction in which the mouth and tentacles point.

Results

Description of the colony

A *Proboscidactyla* colony is composed of a stolon network and three polyp types: gastrozooid, gonozooid (blastostyle), and dactylozooid. Polyps and stolons are patterned axially along the distal portion of a sabellid worm tube.

[1] Present Address: Department of Organismic Biology, University of California, Irvine California 92664.

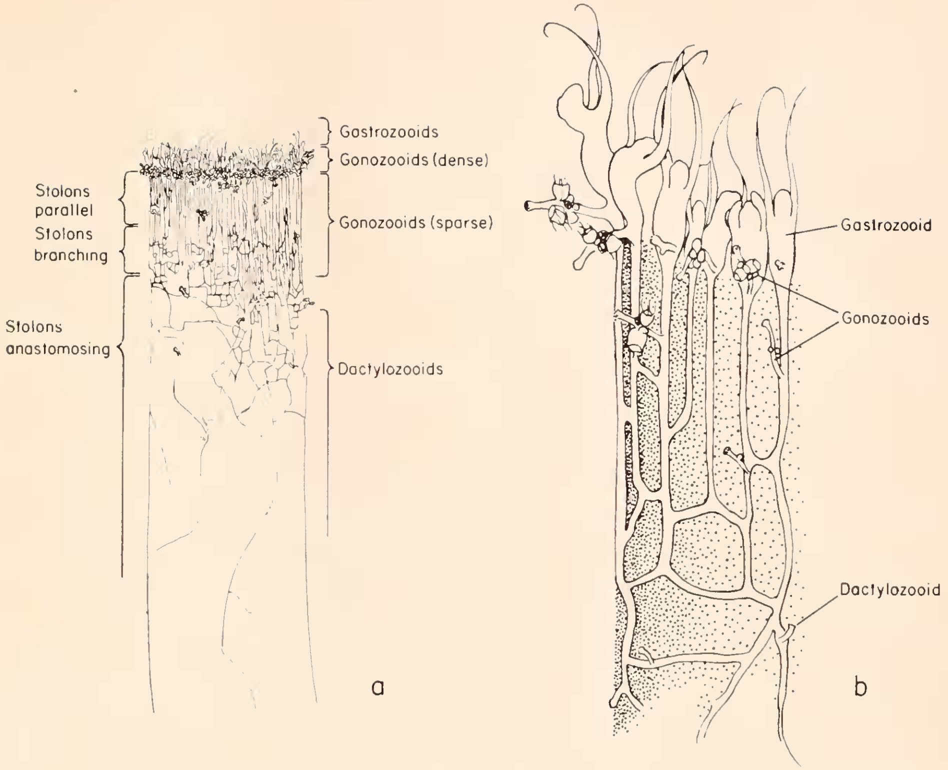

FIGURE 1. *Proboscidactyla flavicirrata* colony pattern. 1a: Axial colonial regions along worm tube. 1b: Polyp insertion and stolon pattern (longitudinal axis greatly shortened).

The gastrozooids are located almost exclusively in a whorl at the rim of the tube (Fig. 1). Gastrozooids are bilateral in structure (Figs. 1b, 3). The curved hypostome inserts vertically on the tip of the arching body column and points in the same direction as the body column arches (Fig. 3), so that the mouth is directed "forward." The two tentacles arising at the base of the hypostome are also directed forward, and a battery of nematocysts is located on the upper side of the hypostome. At the base of the polyp, protruding forward, is a small "foot" (Fig. 3) (see Campbell, 1968a), which may be homologous with the stolon tip of other hydroids. The single stolon connected to each gastrozooid inserts at the base of the polyp "back" (Fig. 3). The stolons (Fig. 1) leading back from the gastrozooids regularly run parallel to the tube axis and alongside adjacent stolons for several millimeters. Further from the tube tip, the stolons become thinner and show increasing branching, thus disrupting the strictly parallel arrangement. In the proximal portions they form an irregular network which becomes indiscernible farther back along the tube.

The gonozooid (Fig. 1), a long column capped by a nematocyst cluster, bears a bouquet of medusa buds midway along its length. Most gonozooids insert on the stolon immediately behind a gastrozooid or on a gastrozooid column. (Almost all

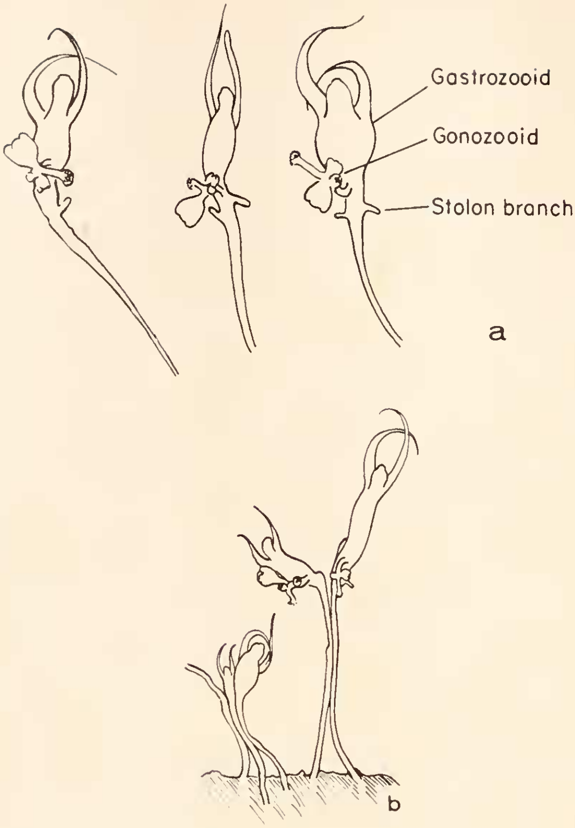

FIGURE 2. Gastrozooids, with gonozooids, migrating along glass tube, 24 hours after colony was streched over the glass. Tracings from photographs. 2a: Three polyps which have stopped migrating, with stolon branches developing behind. These branches resemble those giving rise to new gastrozooids and to stolon anastomosis. 2b: Several polyps showing tendency to follow stolons. Shaded portion represents the edge of the worm tube.

large gastrozooids have such an associated gonozooid.) However, a few gonozooids are scattered sparsely over the distal half of the colony, inserted at random positions on the stolon network.

The dactylozooids (Hirai, 1960) are short, straight or slightly curved polyps, each crowned by a nematocyst bundle. They are scattered over the proximal region of the colony. Uchida and Okuda (1941) have termed such polyps as minute gonozooids lacking medusa buds. Hand (1954) termed them as young or abortive gonozooids.

Intermediate polyp forms may be found between the territories of gonozooids and dactylozooids. These forms are intermediate in length and often bear one or several minute or abortive medusa buds.

The *Proboscidactyla* colony organization is thus predominantly axiate along the tube, with several regions identifiable in terms of stolon patterns and polyp types.

Observations on colony growth and expansion

A. *Overall pattern*

Some colonies were observed for more than two weeks, during which time they grew in length, accommodating elongation of the worm tubes, but did not change in overall pattern. Furthermore, nearly every one of hundreds of colonies studied throughout the summer and at intervals through the winter showed similar organization regardless of the size of the colony and tube (except for colonies on very small tubes, as discussed below). Apparently the colony is in a steady-state developmental pattern, where continuing growth and expansion do not result in a qualitative change in pattern.

FIGURE 3. Gastrozooid, with gonozooid, migrating on a glass microscope slide. Photograph, taken from the side, was printed from a 16 mm. film. Gastrozooid hypostome is in upper right; two tentacles are at lower right. Arrow points to "foot." The stolon (broken white lines) runs off to the left.

B. *Developmental cycle of polyps*

Gastrozooids arise through modification of a stolon tip at the rim of the worm tube. This stolon forms as a lateral branch from one of the parallel stolons just behind the tube rim. It does not transform into a polyp until after it has extended slightly beyond the tube rim. At this time there is a slight flattening of the tip in a plane tangential to the tube surface. Two tentacle rudiments first become visible as broad lateral wings on this stolon tip. As these elongate, their positions shift towards the "front," where they eventually become spaced about 40°–70° from one another. This shifting is probably due to the more rapid growth of the polyp rudiment on the back surface, rather than to an actual movement of the tentacle rudiments across the column tissue. Tissue above the insertion narrows to form a hypostome. When the polyp rudiment has increased its size by several-fold, it has assumed the typical appearance of an older polyp.

Within a single colony, gastrozooids range from these small young individuals to polyps 4 mm. in length. In some colonies there is evidence of a graded distribution of average polyp size from one side of the tube to the other. No stages of gastrozooid degeneration were consistently found. Thus it appears that gastrozooids arise beside, or intercalated between, existing polyps through transformation of stolon tips, and that the polyps then have a relatively long life during which they grow continuously larger.

Gonozooids arise as small buds on the "backs" of the gastrozooids, about midway up the column. Gonozooid buds were found in no other position on the colony. Almost as soon as they are visible the buds are multilobed. As the stalk elongates, the terminal lobe becomes the nematocyst cap, and grows upwards on its own stalk. The other lobes remain in the midstalk region and become medusa buds. The stalk grows relatively rapidly until it is about the same length as the parent gastrozooid. It also changes its position on the gastrozooid, generally moving to the base or just behind the base of the mother polyp.

Gonozooids scattered behind the terminal polyp whorl have few or no medusa buds.

C. *Migratory behavior of gastrozooids*

Gastrozooids are capable of moving across a substratum in a forward direction. This can be demonstrated by extending the polyps' substratum beyond the tube rim, or by explanting polyps onto an appropriate surface under sea water. Under both conditions the gastrozooids will move forward at rates of up to 15 mm. per day.

A simple way to extend the polyps' substratum is to slip the tube over a glass rod. The elasticity of the tube holds the rim tightly against the glass. After 24 hours, many polyps in such colonies will have moved onto the glass (Figs. 2a, b). A single migrating polyp is shown in Figure 3.

The first visible change in a colony after it is stretched over glass is that the "foot" of each gastrozooid swells and elongates. This is apparent after a few hours. Several lines of evidence indicate that this foot directs the subsequent polyp migration. The zooids move forward although not necessarily in a straight line; when their paths curve the feet are also arched. Polyps tend to be guided by physical edges which only the foot may contact; thus, one polyp will follow the stolon of another (Fig. 2b). Finally, if a polyp is cut off from its base, rotated 90° or 180° and grafted back to the base, movement continues in the direction of stolon and foot orientation, not in the polyp's forward direction.

Gonozooids generally move along with the gastrozooid. But occasionally this close relationship is lost and a gonozooid remains isolated on the stolons while the gastrozooids continue advancing.

Mitotic figures (viewed in stained sections of several dozen migrating polyps and their stolons) are absent from the polyp base, but are abundant along the ectoderm and endoderm of stolons behind moving gastrozooids.

Unfed animals move up to several centimeters during the first 36 hours following isolation, but then stop. If fed, polyps migrate for a longer period, but never for more than three days. It is not clear whether food is limiting in these cases; while isolated gastrozooids do diminish greatly in size during movement, some gastrozooids in intact colonies do not.

After migration has stopped, lateral branches are frequently formed from the stolon just behind the gastrozooid (Fig. 2a).

The forward movement of gastrozooids described above is probably involved in the natural growth of the *Proboscidactyla* colony. When worms were grown for five weeks in filtered sea water, the new increments of tube added by the worm were transparent and thus easily distinguished from the previously existing opaque portions. The polyps continued to remain at the rim of the tube, with the entire whorl of gastrozooids eventually located on the transparent tube. Thus movement of polyps along the worm tube occurs during normal growth.

Occasionally, in natural colonies, individual gastrozooids are found away from the tube rim. These are directed in random orientations, their stolons indicating that they had previously been moving parallel to other polyps, but for some reason wandered from the tube rim.

Deduced colonial growth pattern

The observations outlined above suggest a growth pattern by which the entire colony remains in a steady-state condition. As the worm extends its tube at the distal rim, the gastrozooids advance forward, thereby maintaining their terminal positions. The individual polyps thus remain in a strategic ecological position and probably live for a relatively long time.

As gastrozooids advance, the stolon behind the polyps increases in length. This leads to the typical pattern of parallel stolons encircling the distal portion of the worm tube. The stolon system, therefore, is progressively older towards the proximal end of the tube. Apparently the stolons gradually form lateral branches which fuse, leading to progressive anastomosis at greater distances from the tube rim.

Some lateral branches are present in the distal portion. Generally these branches bend forward and extend toward the tube rim between the parent and adjacent stolon. These young stolons are the source of new gastrozooids, their tips being transformed directly into a polyp. Some control process must eventually limit the rate of new gastrozooid formation; possibly high stolon density inhibits lateral branching.

The gonozooids arise on the lower column of the gastrozooid. They remain attached to, or closely associated with, the mother polyp. Occasionally they fall back, are dissociated from the movements of the gastrozooid, and are left behind in the colony. Actually, they still probably continue moving forward to some extent because stolon growth (judging from mitotic figure distributions) occurs along the length of the unbranched stolon portions. These isolated gonozooids gradually lose their medusa buds and diminish in size, becoming dactylozooids.

This model of colonial growth is shown in Figure 4. The colony consists of an active front, which keeps pace with the advancing tube rim, and a stationary

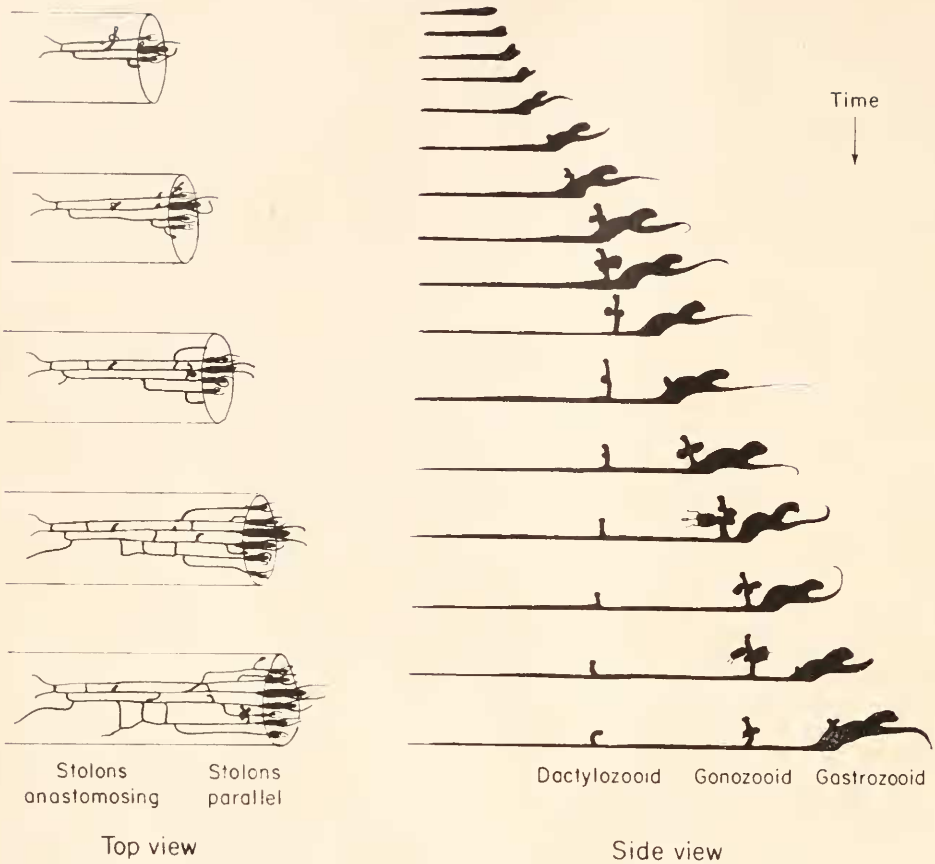

FIGURE 4. Deduced colony progression and development pattern (see text).

network of stolons covering the proximal portion of the worm tube. Individual gastrozooids represent at least relatively permanent components of the active front; they may or may not undergo a gradual replacement cycle. Gonozooids go through a more rapid replacement cycle; individuals occasionally stop advancing with the gastrozooid, stop forming medusa buds, and diminish in size. Each one lost in this manner is replaced by a new one budded from the column of the parent gastrozooid. The stolon system represents the trail of gastrozooids, slowly elaborated by anastomosing.

DISCUSSION

The unique *Proboscidactyla* colonial growth pattern results from the ability of the gastrozooids to move. Colonial succession in other hydroids which have been studied involves extension of the colony boundaries by stolon growth, followed by new polyps arising in the peripheral zones. Older colony regions may die and be resorbed, giving rise to a net displacement of a colony unchanging in size (such as occurs in *Campanularia;* Crowell, 1953) or the older portions may remain active, in which case the colony expands in size (such as in *Podocoryne;* Braverman, 1964). In neither case is there any movement of any portion of the colony relative to the substratum, except for perhaps the terminal portions of the stolons. In *Proboscidactyla*, on the other hand, individual polyps migrate during colony translocation.

This migration pattern shows two points of similarity to the growth patterns of other hydroids. First, the movement of the gastrozooids appears to be similar and perhaps homologous to the elongation of the stolons in other hydroids. According to this view, the "foot" of the *Proboscidactyla* polyp represents the stolon tip; the gastrozooid would therefore be equivalent to a normal polyp which is situated just behind the stolon tip. Spots of vital dye applied near stolon tips in a number of hydroid genera (*Obelia*, Berrill, 1949; *Cordylophora*, Overton, 1963; *Clytia*, Hale, 1964) do move with the tip as does the *Proboscidactyla* gastrozooid (see also Campbell, 1966, 1968b).

Secondly, *Proboscidactyla* polyp movement resembles that of the upright growth in athecate hydroids bearing terminal polyps, where the individual polyp moves away from the colony center through upright elongation. The main difference between the two growth types is the relation of the polyp and stalk to the substratum.

Some descriptions of *Proboscidactyla* colonies do not indicate the presence of a terminal portion where stolons run parallel. These descriptions are of colonies on small worm tubes. I also observed small colonies to lack regular stolon patterns. Probably larger worm tubes elongate more rapidly than small ones. Since stolons do gradually anastomose, one would expect to find parallel cords of stolons during rapid elongation; at these times polyps can advance forward significant distances before stolon branching occurs. This consideration may explain the differences in colony regularity on large and small worm tubes, and the differences in stolon pattern and polyp distributions among related species (see Hand, 1954).

Summary

1. The colony of the hydroid *Proboscidactyla* covers the terminal portions of a sabellid worm tube. The colony is axially patterned. In distal regions stolons run parallel to the form tube axis while in proximal regions they form an anastomosing network. Gastrozooids are situated at the tube rim, gonozooids in a whorl behind gastrozooids, and dactylozooids in the proximal colony regions.

2. Gastrozooids will migrate actively forward when they are not at the edge of a substratum. The gonozooid associated with each gastrozooid generally moves with it.

3. The colony appears to be in a steady-state condition. The whorl of gastrozooids, and associated gonozooids, migrates forward as the tube rim advances due to secretion by the worm. The stolon system is generated behind the advancing gastrozooids. The proximal end of the colony progressively loses its regularity and definition. The axiate patterns of the colony at any moment thus trace out the history of the colony.

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